Thyroglobulin: a thyroid autoantigen and marker of DTC

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Thyroglobulin (Tg) was first recognised as a thyroid autoantigen following the discovery by Roitt and others in 1956 of antibodies to thyroglobulin in the sera of patients with Hashimoto’s thyroiditis. In the ensuing decades it has emerged as a major tool in the follow-up of patients with differentiated thyroid cancer (DTC). Recent progress in recombinant DNA technology has led to the unravelling of its primary structure. However, the contribution Tg makes to autoimmune thyroid disease (AITD) remains unclear and methodological problems with measuring this analyte still pose major challenges to its value in monitoring DTC. This review explores thyroglobulin’s role in AITD and DTC as well as its clinical application as a marker for DTC.

Thyroglobulin (Tg) is the major protein found in the thyroid colloid and is central to thyroid physiology, functioning both as a pro-hormone and a storage site for thyroid hormones. It is a large glycoprotein with a molecular weight of 660,000, and consists of two polypeptide chains each with an approximate molecular weight of 330,000. Thyroglobulin has approximately 2,768 amino acid residues including a 19-amino acid signal peptide. Towards the amino terminal region of the molecule are cysteine-rich repetitive motifs which stabilise the molecule by preventing its degradation by proteases. The major hormonogenic sites are located towards both amino and carboxyl extremities. Towards the C terminal portion of the molecule the amino acid sequence bears a striking similarity with acetylcholinesterase, suggesting a common ancestral gene for both molecules.

Thyroglobulin gene

The human, rat and bovine Tg genes have been determined using recombinant DNA methods. The human gene is located on chromosome 8q 24.2-8q 24.3 and contains more than 30 kilobases of genomic DNA. However, only a small fraction of these bases code for Tg. The remainder make up large intervening intronic sequences. There is a Tg promoter sequence about 170 bases upstream from the transcription-initiating site. This binds the thyroid transcription factor 1 (TTF-1), which is necessary for transcription activation and regulation. Thyroglobulin gene transcription is stimulated by thyroid stimulating hormone (TSH; thyrotropin), while hypophysectomy and T3 treatment decrease transcription. After translation of the mRNA, the formation and export of the mature Tg molecule is a complex process involving the 19-amino acid signal peptide and molecular chaperones such as calnexin, Grp 94 and Bip. Initial glycosylation and folding takes place in the endoplasmic reticulum, with further glycosylation occurring in the golgi apparatus before export to the follicular lumen by exocytosis. This process is supervised by molecular chaperones which ensure quality control in the endoplasmic reticulum by allowing only correctly assembled Tg to be exported.

Thyroglobulin as a thyroid autoantigen

Antibodies to thyroglobulin (TgAb) are found in the serum of patients with autoimmune thyroid disorders (AITD), namely Hashimoto’s thyroiditis (HT) and Graves’ disease (GD). Unlike the thyroid peroxidase antibody (TPOAb), TgAb is not always present in AITD, does not fix complement and has no proven role in the development of AITD. TgAb is also found in healthy subjects without thyroid disease as well as in patients with DTC, and it remains to be proven if healthy individuals with TgAb are at risk of thyroiditis. There is however evidence that TgAb found in healthy individuals differs from that seen in subjects with AITD.

Thyroid hormone synthesis

Iodine is required for normal thyroid hormone synthesis and is available from the diet through iodine-rich foods such as sea fish and iodinated salt, milk or bread. Iodine is actively taken up from the circulation by thyroid epithelial cells in the form of iodide, and then concentrated within the follicular lumen [Figure 1]. The sodium iodide symporter (NIS) of the basal membrane enables active transport of iodide across the basolateral membrane, while pendrin at the apical membrane ensures transport of iodide to the colloid. In the lumen iodide is oxidised by the transmembrane enzyme, thyroid peroxidase, before incorporation into the tyrosine groups of thyroglobulin as monoiodotyrosine (MIT) and diiodotyrosine (DIT) residues. These are subsequently coupled in the presence of thyroid peroxidase to yield T3 and T4 which are then secreted by endocytosis of thyroglobulin.
withAITD. Generally, TgAb in healthy subjects are of lesser concentration thanAITDTgAb. In addition, TgAb in pathological states and in healthy subjects appear to react differently with antigenic sites on Tg. Several groups have characterised the TgAb epitope recognition properties using competitive inhibition studies. Such studies employ panels of labelled Tg monoclonal antibodies (Tg-Mabs) which compete with TgAb in test sera for Tg binding sites. Using this approach it has been possible to study the epitope recognition patterns in various disease states as well as in healthy individuals with high TgAb levels. Despite variations in methodology, most of these studies concur that TgAb in subjects withAITD recognise a restricted number (2-3) of Tg epitopes, while TgAb in healthy subjects react with the epitopes in a heterogeneous manner. The epitope recognition pattern in subjects with DTC has given conflicting results.

**Thyroglobulin as a marker of DTC**

In the past few decades, measurement of serum Tg has emerged as an important tool in the postoperative monitoring of patients with DTC. Following surgery for DTC, the presence of Tg in serum implies recurrent or residual disease while absent Tg levels are seen in disease-free individuals. In fact serum Tg has proved to be more sensitive than whole body scans in detecting persistent or recurrent disease.

Radioimmunoassay (RIA) and Immunometric assays (IMA) are routinely used to measure Tg in serum and both methods have comparable sensitivities. Tg RIAs were introduced in 1973 and were later suggested as a marker of differentiated thyroid cancer. They have the disadvantage of long incubation times, limited working ranges and short shelf lives common to radioisotopes. IMAs became popular with the advent of monoclonal antibody techniques and despite the introduction of newer more sensitive immunometric assays (IMA) using monoclonal antibodies, there are still major problems with the accuracy of Tg estimations [Table 1]. Despite the availability of a Tg-standard from the community bureau of reference in most laboratories, differences in antibodies used in the various assays still account for poor inter-laboratory standardisation. In addition, poor inter-assay variation is a potential problem in patients on long term follow up. As a result, it is recommended that the same laboratory and assay method be used for serial serum Tg estimations. Measuring stored patients’ serum with fresh follow up sample and adjusting for inter-assay errors helps to overcome this problem.

Furthermore, thyroglobulin assays may suffer from low functional sensitivity i.e. they are unable to distinguish the lower limit of normal euthyroid range from the functional sensitivity limit of the assay. At the other extreme, very high Tg levels may not be detected by IMAs because of the hook effect. This is due to the presence of large amounts of antigen which exceed the binding capacity of the Tg capture antibody on the solid support. The hook effect can be overcome by diluting the specimen before measurement and it is recommended that serum samples routinely be measured at 2 dilutions.

TgAb interference with Tg estimations is the most common problem with Tg assays. This is quite a significant problem since DTC patients have a higher prevalence of TgAb (20-45%) than the normal population (4-27%). The factors determining interference are unclear since there is poor correlation between TgAb concentration and the degree of interference. Generally, with RIAs there is positive interference resulting in falsely elevated levels. The degree of interference depends on the affinity and specificity of the antibodies used. With IMAs on the other hand, TgAb binds Tg, blocking it from participating in the two-site reaction and thus causing negative interference and falsely low values. Falsely elevated values may cause unnecessary investigation and intervention, while falsely low values may result in the potentially more serious scenario of missing recurrent or metastatic disease.

Several approaches have been tried in an effort to overcome TgAb interference. Monoclonal antibodies restricted to Tg epitopes apparently not recognised by TgAb have been designed for use in IMAs. However, interference is still seen using these monoclonal antibodies. *In vitro* tests of recovery are used by some laboratories, but are technically laborious and do not often give consistent results. Dual estimates of Tg by RIA and IMA for Tg discor-

**Recombinant human TSH (rh-TSH) in DTC**

A remarkable development in the application of serum Tg measurements in the follow up of DTC has been the introduction of recombinant human TSH (rh-TSH) inspiration prior to testing. The sensitivity of serum Tg measurements in the follow up of DTC is improved by withdrawing T4 treatment before testing. However, this causes symptomatic hypothyroidism. The introduction of recombinant human TSH (rh-TSH) stimulation before testing has improved the sensitivity of Tg values in the suppressed state, avoiding hypothyroidism from T4 withdrawal. rh-TSH stimulated Tg measurements compare favourably with thyroid hormone withdrawal Tg testing. A patient with undetectable basal serum Tg and no response to rh-TSH most likely has no remaining thyroid tissue, while a minimal response suggests the presence of minimal thyroid tissue. A substantial response to rh-TSH implies the presence of a well-differentiated tumour and such a patient will not benefit from radioiodine imaging. Other possible uses of rh-TSH in the treatment of DTC and nodular goitre have recently been reviewed.

**Conclusions**

Thyroglobulin is a thyroid autoantigen central to thyroid physiology and hormone synthesis. Despite breakthroughs in the unravelling of the primary sequence of its gene and structure, there are still considerable gaps in our understanding of the contribution Tg makes toAITD. Tg is a major tool in the postoperative monitoring of patients with DTC. However, its value in this regard is limited by problems with antibody interference. On the other hand, the use of rh-TSH stimulation prior to testing has improved the sensitivity of Tg values in the suppressed state.

**Further reading**

Table 1. Major problems encountered with thyroglobulin assays.

- Poor inter-laboratory standardisation
- Poor inter-assay variation
- Low functional sensitivity
- Hook effect
- Thyroglobulin interference

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